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COMMUNICATION

Light-triggered syneresis of a water insoluble peptide-hydrogel effectively removes small molecule waste contaminants†

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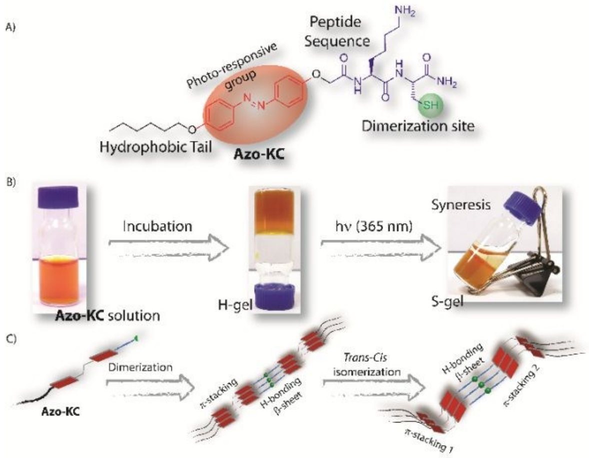
A short peptide based hydrogel exhibits aqueous insolubility, thixotropy and efficient light induced syneresis. Upon irradiation with UV light, the hydrogel shrinks and expells ~ 50% of the solvent. Syneresis is caused by light-triggered *trans-cis* isomerisation of an azobenzene moiety in the peptide derivative. This expulsion of solvent can be effectively exploited in the removal of low molecular weight contaminants in water.

Supramolecular hydrogels made of small peptides have been attracting interest for several decades on account of potential applications in areas such as drug delivery, materials science, sensing and nano-technology to name a few.[1-4] These systems have been rendered stimuli responsive through the introduction of pH, heat, metal ions and light.[5-8] Various supramolecular hydrogels have also been reported with unique properties including aqueous insolubility, stretchability, injectability, unusual aging and strength.[9-13] However, reports regarding syneresis or macroscopic volume phase transitions in response to stimuli are rare for supramolecular gels. Syneresis is the expulsion of liquid from a gel; a common example is the discharge of blood serum during the clotting process.[14] Relatively few examples of supramolecular gels exhibiting syneresis have been reported to date.[15-18] These hydrogels exhibited contraction and expulsion of water in response to various stimuli such as, pH,[19] metal ions,[18] light,[17] mechanical force[16] or heat[17]. Such a property can lead to various important applications in the field of material science and bio-medical field. While an effort was made by Adams and co-workers to propose a rational behind such behaviour in a supramolecular gel,[20] it is still not clear why and when such property could be expected. Probably, all the reported hydrogels are serendipitous observations and a proper model could be developed to design such gelators only with the help of more examples and analyses.



Scheme 1. A) Chemical structure of Azo-KC. B) Photographs of the Azo-KC solution, hydrogel formed by the solution (H-gel) and syneresis (S-gel) upon irradiation with UV light. C) Possible molecular arrangements corresponding to different states as mentioned in (B).

In this regard, we have recently reported a peptide [9, 21] capable of forming hydrogels, which remain insoluble in bulk water and exhibited an unusual property as exchange of solvent and solute to and from these hydrogels is highly restricted. Dimerisation of the gelator was found to be critical for this unique property. Exploring the possibility of creating new hydrogelators with similar effect, we have prepared an azobenzene-functionalised short peptide, **Azo-KC** (Scheme 1), which efficiently formed hydrogels under basic conditions. In addition to insolubility, the hydrogel also displayed light-triggered syneresis. Additionally, the hydrogel found to be responsive toward disulphide-bond breakers and thixotropic property. The combination of all these properties like, dual responsiveness, syneresis, insolubility and injectability make the hydrogel distinctive amongst all such supramolecular hydrogel reported so far. Here, we report the hydrogelation by **Azo-KC**, its aqueous insolubility, light-driven syneresis and its ability to effectively remove contaminants from water.

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An aqueous solution of **Azo-KC** forms a self-supporting hydrogel (H-gel, Scheme 1) upon treatment with base (NaOH). The minimum gelation concentration (MGC) and gel to sol transition temperature at MGC were found to be 1.15 wt% (16.2 mM of **Azo-KC**) and 57 °C, respectively. Transmission electron microscopic (TEM) analysis shows formation of a network of thin fibers (2-5 nm × ~100 nm; Fig. 1A). Rheological measurements confirmed a gelled state of the material with a higher storage modulus (G') compared to its loss modulus (G") in both amplitude and frequency sweeps (Fig. 1B and S1).[22] While performing the strain-dependent rheology measurement, the hydrogel showed a yield strain and transforms into a quasi-liquid indicating possible thixotropic (injectable) behaviour.[23] A time-dependent strain sweep experiment was performed by alternating the applied strains at a fixed angular frequency. At a higher strain (γ = 1000%), the viscoelastic properties of the material were lost, but were regained almost completely in every successive steps when a lower strain (γ = 0.1%, Fig. 1C) was applied. This step-strain measurement was cycled four times with excellent recovery, demonstrating thixotropic behaviour by the hydrogel. The hydrogel could easily be injected through a syringe immediately recovering as a gel after shear (Fig. S2).

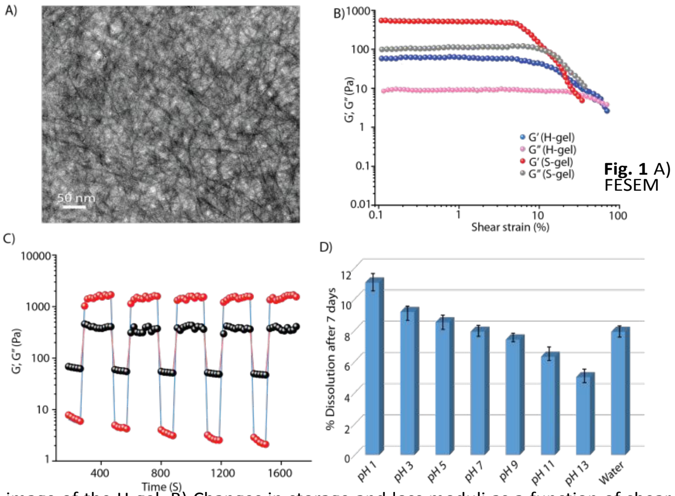

Fig. 1 A) FESEM image of the H-gel. B) Changes in storage and loss moduli as a function of shear strain for H- and S-gels. C) The rheological properties of 1.15 wt% H-gel when alternate step strain switched from 1% to 200%. D) % dissolution of the H-gel in buffers of different pH (1-13) after 7 days of incubation at room temperature.

It is important to note that, unlike cross-linked polymeric hydrogels, the formation of supramolecular hydrogel networks are entirely governed by non-covalent weak forces. Thus, when dispersed in bulk water, these networks disassemble and the individual gelator molecules become solubilised which results in an overall gain in entropy of the system. However, in line with our previous report,[9] the hydrogel formed by **Azo-KC** was also found to be insoluble in water. A small portion of the hydrogel was placed in bulk water or buffers at different pH values (1-13) and shaken at a constant speed. The bulk medium was analysed over time using UV-Vis spectroscopy to quantify the dissolution (Fig. S3-4). In all media, an initial dissolution (up to 11%) was observed, corresponding to loosely-bound gelator molecules at the surface of the hydrogel. However, in all cases, after 24 h no further dissolution was observed. The extent of dissolution in these media after 7 d are shown in Fig. 1D. The hydrogel remained insoluble even after one year of incubation in bulk water.

The self-assembly of **Azo-KC** is assisted by the disulphide bridge between two peptides. After 2 h of incubation under basic conditions, only the mass corresponding to the **Azo-KC** dimer was found. Time-dependent high pressure liquid chromatographic (HPLC) analyses of a diluted solution of **Azo-KC** shown in Fig. 2A revealed that the dimerisation is complete within 1.5 h. The highly basic medium in this case presumably facilitates oxidation of the Cysteine thiols.[21] To understand the situation in the gel state, a portion of the H-gel was dissolved in DMSO and further diluted with water and analysed using analytical HPLC. More than 99% of the molecules were found to be in the dimer form (Fig. S5). To demonstrate the importance of the disulphide linkage towards gelation, the hydrogels were placed into aqueous solutions containing various disulphide bond-breaking agents. In all cases the hydrogel dissolved within minutes (Fig. S3).

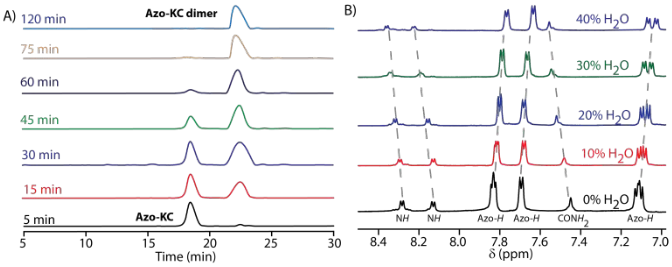

Fig. 2 A) Time dependent chromatographic analysis of a 0.01 wt% aqueous solution of **Azo-KC** showing the formation of disulfide linked dimer. B) ^1H NMR spectra of **Azo-KC** dimer in DMSO-d_6 with varying amounts of H_2O showing the down-field shifts of the amine N*H*s and up-field movements of the aromatic protons. All measurements were carried out at room temperature.

A ^1H NMR was carried out on solutions of **Azo-KC** in DMSO-d_6 after 12 h with varying amounts of water. This revealed that both hydrogen bonding and π–π stacking interactions become prominent in the aggregated state.[24] With an increase in water content, the amide-N*H*s showed down-field shifts while the aromatic protons moved upfield (Fig. 2B). Powder XRD analyses of the as synthesised **Azo-KC** and xerogel obtained from the H-gel confirmed π–π stacking between the "Azo" groups (interplaner distance of 4.23 Å) in the self-assembled state (Fig. S6). Fourier-transform infrared (FTIR) spectra of the as synthesised peptide showed two peaks at 1683 and 1652 cm^{-1}, which were shifted to 1673 and 1639 cm^{-1}, respectively in the H-gel (Fig. S7). The observed shifts and peak positions in the H-gel indicate possible hydrogen bonding between the neighbouring peptide molecules.[25]

The gelator, **Azo-KC**, was designed to embed a light sensitive moiety into a hydrogel by incorporating an "Azo" group, which can undergo *trans* to *cis* isomerisation upon irradiation with UV light.[17, 26] When irradiated with light (λ = 365 nm), the H-gel displayed shrinkage, expelling water from the hydrogel (Fig. 3A). This unexpected observation lead us to perform a systematic evaluation of hydrogel syneresis. Quantitative analysis showed that the hydrogel can shrink up to maximum of 50% of its original volume following irradiation with UV light (Fig. 3A). It is clear from the plot in Fig. 3A that the shrinkage process is complete within 90 min. HPLC (Fig. S5) and ESI-MS

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analyses of the shrunken gel (S-gel, Scheme 1, Fig. 3A) confirm that the constituent molecule of S-gel remains the disulphide linked dimer of **Azo-KC**. The HPLC analyses shows ~ 9:1 distribution of *cis* and *trans* isomers of the dimer in the S-gel sample.

To check reversibility of the syneresis process, the shrunken gel was further irradiated with light of 420 nm as well as kept in direct sunlight for several hours with occasional shaking. However, no change in the appearance was observed, which confirmed irreversibility of the process. Water expulsion was also monitored by keeping the H-gel at room temperature for several days. However, no syneresis was observed in this case. Finally, the hydrogel was heated at 45 °C and only a small portion (~ 15%) of the total water was expelled from the hydrogel after 2 h of heating. Thus, though heating can cause syneresis in this system, UV light irradiation was found to be a more effective stimulus.

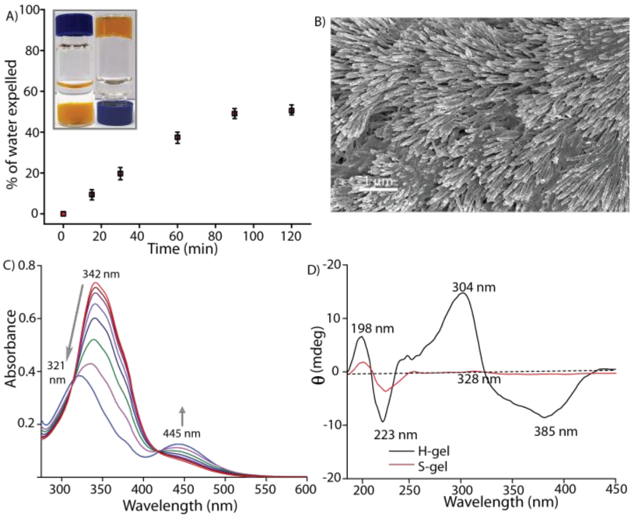

Fig. 3 A) % of water expelled from the H-gel during syneresis as a function of duration of the UV-irradiation process. Inset: images of the vials after 2 h of irradiation as in (A) showing the expelled water. B) FESEM image of the S-gel. C) UV-Visible spectra of a 0.01 wt% 12 h matured aqueous solution of **Azo-KC** (in presence of NaOH) during UV-irradiation. D) CD spectra of a 0.01 wt% 12 h matured aqueous solution of **Azo-KC** (in presence of NaOH) before and after 2 h of UV-irradiation. All measurements were carried out at room temperature.

The syneresis observed in this system is an interesting phenomenon, thus gaining mechanistic insight into the system is important for enhanced understanding. The S-gel was analysed using electron microscopy as well as rheology. Interestingly, the S-gel consists of ~ 1 μm long rod-like structures (Fig. 3B). This morphology clearly differs from the original network of thin fibers observed for the H-gel. The sample after 30 min of exposure to UV light shows a combination of both fibers and rods, which reflects an intermediate state of the morphogeneses process (Fig. S8). For the S-gel, both storage and loss moduli were found to be much higher than that of the H-gel (Fig. 1B and S1). These results suggest a significant alteration takes place in the self-assembly of the gelator molecules upon irradiation with UV light.

The light induced "*trans*" to "*cis*" structural isomerisation of **Azo-KC** changes the self-assembly state. The UV-Vis absorption spectra of a solution of **Azo-KC** dimer was recorded at different time intervals while irradiating the solution with light (365 nm). Before irradiation, the molecule showed an absorption maxima at 342 nm (Fig. 3C), which corresponds to the π–π transition of the *trans*-azobenzene unit.[27] As the sample was irradiated with UV light, a hypsochromic shift was observed in the absorption maxima. After 90 min of irradiation, the absorption maxima was 321 nm and the molar extinction coefficient decreased linearly for the respective absorption maxima. Additionally, a new broad peak at 445 nm appeared in the irradiated sample increasing with irradiation time. This new peak is a signature of the *n*–π* transition of *cis*-azobenzene.[27]

Circular dichroism (CD) of the **Azo-KC** hydrogel revealed a bisignate signal in the azobenzene absorption region with a positive to negative crossover point at 324 nm (Fig. 3D). Additionally, a positive peak at 198 nm and a negative Cotton effect at 222 nm were also observed indicating β-sheet like arrangement of the peptide. The strong exciton-coupled cotton effect at the bisgnate peak is a result of supramolecular chirality in the aggregated state.[17] While the secondary conformation of the peptide remained similar for the S-gel, the 250-400 nm region remained CD silent. PXRD of the xerogel formed from the S-gel showed two π–π stacking interactions (4.16 and 3.79 Å) compared to only a single peak for the H-gel (Fig. S6). However, the hydrogen bonding interactions are still observed in the IR spectra with two prominent bands at 1677 and 1637 cm^{-1} supporting a β-sheet like arrangement similar to that of the H-gel (Fig. S7).

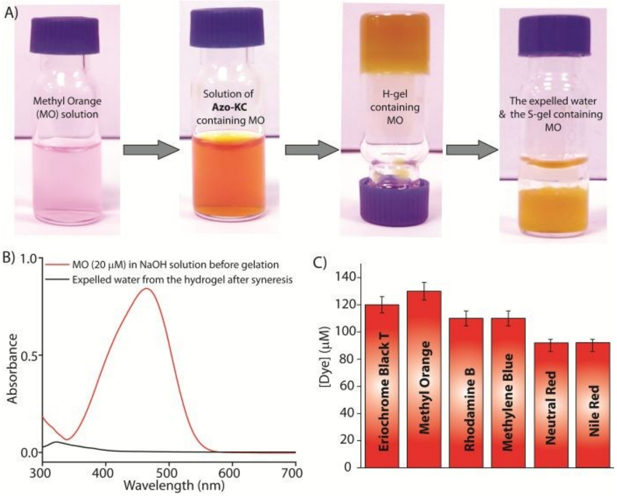

Fig. 4 A) Photographs showing MO removal from H-gel upon UV light induced syneresis. B) UV-Vis spectra of MO and the expelled water from (A). C) Maximum initial dye concentrations for different dyes, which can be completely removed through syneresis of the hydrogel. All measurements were carried out at room temperature.

It is important to note that the transformation in morphology occurs while the system is in an aggregated gel state where the dynamics of the constituent molecules are highly restricted.[21] Crystal to crystal transformation due to photo-induced *trans-cis*

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isomerisation of azobenzene derivatives has been reported in literature.[28] However, such gel-state morphogenesis are not commonly observed phenomenon.[29] It is clear that during the *trans-cis* isomerisation, the **Azo-KC** dimer undergoes a significant change in molecular arrangement, which is shown in Scheme 1. Presumably the new arrangement in the S-gel requires less water to stabilise the aggregated structure. Thus, the system expels excess water present in the system. Moreover, when exposed to the disulphide bond breaking agents, the S-gel did not get dissolved. This observation also suggests much stronger packing of the molecules in the S-gel compared to that in H-gel. However, determination of the exact molecular arrangement in the shrunken state requires further detailed analyses.

We anticipated that light-triggered shrinkage of the H-gel, could be used to entrap toxic small molecules, including dyes, in the S-gel structure. Six different model dyes were tested. H-gels (at MGC) were prepared in 20 μM solutions of the model dyes. The H-gels were then subjected to syneresis by irradiation with UV light (365 nm) for 2 h. The expelled water was then analysed using UV-Vis spectroscopy to quantify the concentration of dye expelled. Importantly, the expelled water was found to be free of dye in all cases. Fig. 4A-B show the results for methyl orange (MO) while the results of other dyes are reported in the ESI (Fig. S9-10). Keeping the gelator concentration fixed at MGC, different initial concentrations of the dyes were then evaluated. For MO, the syneresis process could remove the dye completely at or below 120 μM initial dye concentration as analysed by UV-Visible spectra (Fig. 4B) and analytical HPLC (Fig. S10). Above this concentration, a small amount of MO was found in the expelled water. For other dyes, the results were similar and are shown in Fig. 4C.

In summary, we have presented a small peptide based hydrogel, which displayed insolubility in water as well as buffers of different pH (pH 1-13). The hydrogel was found to be thixotropic in nature and exhibited efficient syneresis upon irradiation with UV light. The syneresis is stimulated through light-induced *trans-cis* isomerisation of the gelator molecule, which rearranged within the gelled state and expel the excess unwanted water. The syneresis process was successfully utilised to efficiently remove model dyes from water.

Conflicts of interest

There are no conflicts to declare.

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Syneresis: A water insoluble hydrogel that expels 50% of the water upon irradiation with UV-light.